Tracing of mono- and polysynaptic afferent connections between the main olfactory bulb and higher-order brain regions in the mouse

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ABSTRACTS

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KEYNOTE LECTURES

K1 - Deconstructing Smell.

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The mammalian olfactory system possesses immense discriminatory power. It can generate diverse odor perceptions to a vast array of volatile odorants and stimulate instinctive behaviors and hormonal changes to conspecific and interspecific social cues. Our work has focused on two questions. First, how do mammals detect so many different chemicals. And second, how does the nervous system translate those chemicals into diverse perceptions and innate responses. Using a combination of molecular, cellular, and genetic approaches, we have identified four different families of chemosensory receptors expressed by sensory neurons in the olfactory epithelium and vomeronasal organ and interrogated how signals from different receptors are organized and used to encode chemical identities in these peripheral sense organs and in the main olfactory bulb. Our recent studies indicate that neurons expressing odorant receptors (ORs) and TAARs belong to different lineages of nasal sensory neurons and that distinct nuclear locations of these receptors may underlie their segregated expression. We are currently using viral tracers and pharmacogenetic methods to gain insight into the neural circuits underlying instinctive responses to social cues.

K2 - Taste Cells of the Gut and Endocrine Cells of the Tongue.

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We have found that many of the receptors and downstream signalling elements involved in taste detection and transduction are expressed also in intestinal hormone producing (endocrine) cells where they underlie key chemosensory functions of the gut. In one example of gastrointestinal chemosensation it is known that glucose given orally, but not systemically, induces secretion of the "incretin" hormone GLP-1 (glucagon like peptide-1), which in turn regulates insulin secretion and glucose homeostasis. We have found that intestinal endocrine cells express sweet taste receptors, the taste G-protein gustducin, and several other taste transduction elements. Knockout mice lacking gustducin or the sweet taste receptor subunit T1R3 have deficiencies in secretion of GLP-1 and in the regulation of plasma levels of insulin and glucose. In another example of gastrointestinal chemosensation we have found that endocrine cells of the pancreas express multiple taste proteins that are involved in regulating insulin release. Furthermore, taste cells of the oral cavity express GLP-1, other "gut" hormones and the insulin receptor. Recently, we identified intestinal-type glucose transporters and pancreatic-type ATP-gated K+ channels (K-ATP metabolic sensors) as being present in taste cells and potentially functioning in the detection of the sweet taste of sugars. Most recently, we have found that the intestinal brush border disaccharidases maltase glucoamylase and sucrase-isomaltase are expressed in T1R3+ taste cells. Further, we found that two disaccharidase inhibitors significantly reduced gustatory nerve responses to sucrose and maltose, but not to the monosaccharides glucose and fructose or the noncaloric sweeteners. We propose that these enzymes act in concert with salivary amylase to generate free glucose from sucrose, maltose and starch that can activate the T1R3-independent sugar detection pathway. In sum our studies point out similarities in gustation and gut chemosensation and indicate the importance of "taste cells of the gut" and "endocrine cells of the tongue" in coordinating the body's hormone responses to regulate glucose homeostasis.

K3 - Olfactory Circuits: From Receptors to Behavior.

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In the mouse olfactory system, individual glomeruli in the olfactory bulb (OB) represent a single type of odorant receptor (OR). A predator odor, TMT (trimethyl-thiazoline), activates multiple glomeruli in both the dorsal and ventral regions in the OB. However, a limited number of glomeruli localizing to the posterior DII region (J domain) are responsible for

the contribution of various types of K+ channels which are likely to get activated during Nav-mediated depolarization. Although blockade of Kv4 and Ca2+-activated K channels did not interfere with (ΔF/F)TPU and uEPSPs, a NEURON model supports the indirect evidence of a slowly repolarizing current that is Nav-activated and fits the properties of delayed-rectifier potassium channels (KDR). Our results suggest that the local Nav mediated mechanism may endow the olfactory bulb with an extra mode of computation that serves to fine-tune the primary representation of olfactory sensory stimuli. The observed acceleration of EPSPs by Navs may contribute to precise timing of GC output, e.g. in the context of fast sensory-evoked network oscillations.

P58 - Hierarchical Deconstruction of Olfactory Sensory Neurons: from Whole Organ to Single-Cell RNA-seq.

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The mouse olfactory mucosa is a complex chemosensory tissue composed of multiple cell types, neuronal and non-neuronal. We have here applied RNA-seq hierarchically, starting with crude tissue samples dissected from the nose, proceeding to flow-cytometrically sorted pools of mature olfactory sensory neurons (OSNs), and finally arriving at single mature OSNs. We show that 98.9% of intact olfactory receptor (OR) genes are expressed in mature OSNs, and uncover a hitherto unknown bipartition among mature OSNs. We find that 19 of 21 single mature OSNs each express a single intact OR gene abundantly, consistent with the one neuron-one receptor rule. Monoallelic expression of this abundantly expressed OR gene is extremely tight. The remaining two single mature OSNs appear to be examples of type B Trpc2+ cells in the main olfactory epithelium, which we here establish as a neuronal cell type that is fundamentally distinct from canonical OSNs.

P59 - Tracing of mono- and polysynaptic afferent connections between the main olfactory bulb and higher-order brain regions in the mouse.

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Processing of odors in the main olfactory bulb (MOB) is modulated by higher brain afferents depending on the internal state, motivation, memory and emotions. For example satiety or hunger are known to change the perception of food odors. To shed light on this modulation a greater understanding of the underlying circuitry is required. To this aim we conducted tracing experiments in mice. First, stereotaxic injections of monosynaptic retrograde DiI and choleratoxin subunit B (CTb) into the dorsal olfactory bulb (focusing on the granular layer) were performed. In a second approach, we injected the pseudorabies virus 152 (PRV152) (kindly provided by Prof L. Enquist; Princeton University). As a neuronal tracer, this neurotropic virus can spread in synaptically connected neurons, dissecting the entire circuitry. The temporal analysis of the viral distribution allows to determine the number of synapses crossed. Both DiI and CTb confirm all the main centrifugal afferents to the MOB which were previously described in the literature. Thus, except the olfactory tubercle, all regions belonging to the olfactory primary cortex were labeled. Moreover, in the piriform cortex, labeling was mainly located in its dorsal part, confirming the topographical anatomical organization of cortico-bulbar projections. Direct projections arising from orexinergic neurons in the lateral hypothalamus were also observed. Regarding polysynaptic tracing, mice were sacrificed one, two and three days after PRV152 injections. After one day, only some of the direct neuromodulatory afferents were labelled. Thus, the locus coeruleus, which has a very caudal location in the brain close to the forth ventricle, showed already staining. In contrast, other neuromodulatory afferents (arising from raphe nuclei, ventral tegmental area, basal forebrain) were not labeled at this stage. Two days after injection, comparison with DiI/CTb results indicate that all the first-order connections were labeled. At this stage, second-order projections started to appear (for example, the midline thalamic nuclei as reuniens and rhomboid nuclei). Lastly, after three days, an extensive brain labeling occurred although some brain regions still showed a lack of staining. In conclusion, using DiI/CTb tracers that do not cross synapses allow to stain all primary afferents. In contrast, viral tracer migration depends on timing as well as number of connecting synapses. Thus, labeling occurring already one day after injection indicates a strong connection. Accordingly, our results suggest that the stained cells in the locus coeruleus send strong projections to the MOB. Furthermore this method allows to follow up the circuits involved in olfactory modulation. Data analysis of all the other labeled regions, focusing on hypothalamic nuclei and brain areas involved in arousal and food intake, are in progress.

P60 - Multimodal priming effects of facial expressions as visual stimuli on olfactory perception and cognition - an fMRI study.

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